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Water quality and biotic interaction of two cavefish species: *Typhleotris madagascariensis* Petit, 1933 and *Typhleotris mararybe* Sparks & Chakrabarty, 2012, in the Mahafaly Plateau groundwater system, Madagascar

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Abstract

The karstic subterranean aquatic system of the Mahafaly Plateau in south-western Madagascar is inhabited by two species of cavefish: *Typhleotris madagascariensis* and *Typhleotris mararybe*. Knowledge about both cavefish species is scant. In order to learn more about the distribution of the two species, 15 caves and sinkholes spread over the Mahafaly Plateau were inventoried for their presence. Abiotic water quality and interspecific relations of the two species were investigated in six of these caves and five of the sinkholes during the dry and the rainy seasons. *Typhleotris madagascariensis* was present in all sampled water bodies while *T. mararybe* was restricted to five sites in the region around the town of Itampolo. The inventories extend the known range of both species of *Typhleotris* on the Mahafaly Plateau. Abiotic water characteristics did not differ between seasons. The abundances of both species were negatively correlated with iron concentrations. Further correlations between the abundance of either fish species and abiotic water characteristics remained inconclusive as these water characteristics co-varied with geographical latitude that in turn was correlated with fish abundance. For both species neither the abundance nor a condition factor based on body mass showed any significant seasonal variation. Also the presence of *T. mararybe* had no influence on the abundance and the condition of *T. madagascariensis*. Thus, no evidence for competition was noticed between the two species.

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Keywords
Mahafaly Plateau, limestone, subterranean water, cavefish, water quality

Introduction

Madagascar is known for its exceptional biodiversity and human reliance on goods and services provided by the original ecosystem, making it one of the world's most prominent biodiversity hotspots (Myers et al. 2000). The island is characterized by high levels of endemism for many groups of organism and a depauperate freshwater ichthyofauna (Benstead et al. 2003, Proudlove 2010). Since 1933, four endemic troglobites fish species have been described from Madagascar: *Glossogobius ankaranensis* Banister, 1994 (Gobiidae) and *Typhleotris madagascariensis* Petit, 1933, *Typhleotris pauliani* Arnoult, 1959 and *Typhleotris mararybe* Sparks & Chakrabarty, 2012 (Eleotridae).

The genus *Typhleotris* is restricted to a vast network of subterranean limestone (karst) habitats in arid regions of coastal south-western Madagascar (Sparks and Chakrabarty 2012). *Tythleotris pauliani* occur in the subterranean waters of Andalambezo-Morombe (Arnoult 1959) north of Tuléar while *T. madagascariensis* and *T. mararybe* are known from the Mahafaly Plateau groundwater network south of Tulear. This plateau extends over 150 km between the the Onilahy river in the north and the Menarandra river in the south (André et al. 2005) and ends in the west with a cliff of up to hundred meters, followed towards the sea by an alluvial plain of sand and sandstones (Dobrilla 2014). Many caves and sinkholes are present in the coastal plain and the western part of the plateau. Some of them end with subterranean lakes populated by blind cavefish. A total of six caves are known to be inhabited by *T. madagascariensis*. This species' geographic range extends from wells near Ambilailalike, located about midway between Soalara and Efoetse in the north, to the sinkhole of Nikotsy near the town of Itampolo in the south (Kiener 1964, Sparks and Chakrabarty 2012). For *T. mararybe* the type locality and only known occurrence is the «*grotte de Vitany*», a sinkhole located near Itampolo on the coastal plain (Sparks and Chakrabarty 2012).

Knowledge of the cavefish fauna from the Mahafaly Plateau is scant. Published studies focussed on species descriptions with very few data on habitats. Most research was restricted to the type localities. Thus, much basic information of Malagasy blind sleeper gobies of the genus of *Typhleotris* is yet to be learned, especially about the subterranean habitat, their distribution, biology and ecology. In order to fill this gap, we inventoried several caves and sinkholes within the Mahafaly Plateau from October to November 2012 (dry season) and from February to April 2013 (rainy season) for the presence of *Typhleotris* spp. and measured physical aspects of water quality. Specifically, we asked the questions:

What are the distributions of *T. madagascariensis* and *T. mararybe*?
Is their occurrence related to abiotic characteristics of water quality?

Is there any evidence for competition between the two species as indicated by changes in morphology in the case of sympatry versus allopatry?

Materials and methods

Study area

Field surveys were conducted in the Mahafaly Plateau, in south-western Madagascar. This karst plateau is characterized a sub-arid climate with low precipitation (Battistini 1964). Annual precipitation varies from 150 to 300 mm in the coastal plain and between 500 and 700 mm on the plateau with extreme inter-annual variation (Juberthie and Decu 2001, Ratovonamana et al. 2013).

In addition to the occurrences of the genus *Typhleotris* reported by Petit (1933), Kiener (1964) and Sparks and Chakrabarty (2012), some subterranean waters known by local people as cavefish habitat were visited during this study. In total, 15 subterranean water bodies were checked for cavefish presence, 11 of them (5 sinkholes and 6 caves) were chosen for measurements of water quality and more detailed fish inventories because of accessibility (Fig. 1).

Water characteristics

Water sampling was carried out on two to four successive days during the dry season and during the rainy seasons at around 9 am. Eleven water physico-chemical parameters that can affect fish population were assessed for each study site. Measurements were performed directly in the field using portable meters. Temperature, dissolved oxygen and oxygen saturation were measured with a Voltcraft DO-100 dissolved oxygen meter. The pH, the electrical conductivity (EC) and total dissolved solids (TDS) were assessed with an HI 98129 electronic pH and EC meter of Hanna. Ammonia, nitrate, nitrite, phosphate and iron analyses were performed using specific reagents and the HI83205 photometer of Hanna. More details on the analytical methods are provided by Rasoloariniaina et al. (2015).

Fish sampling

Fish sampling was carried out seasonally during four successive days. Cavefish were caught for three hours a day using a triangular *Zebco* landing net and an aquarium net. The landing net was a 60×50×60 cm bow with adjustable handles (up to 2 m) and 6 mm mesh size. The aquarium net had a plastic coated metal handle and 200 micron mesh size.

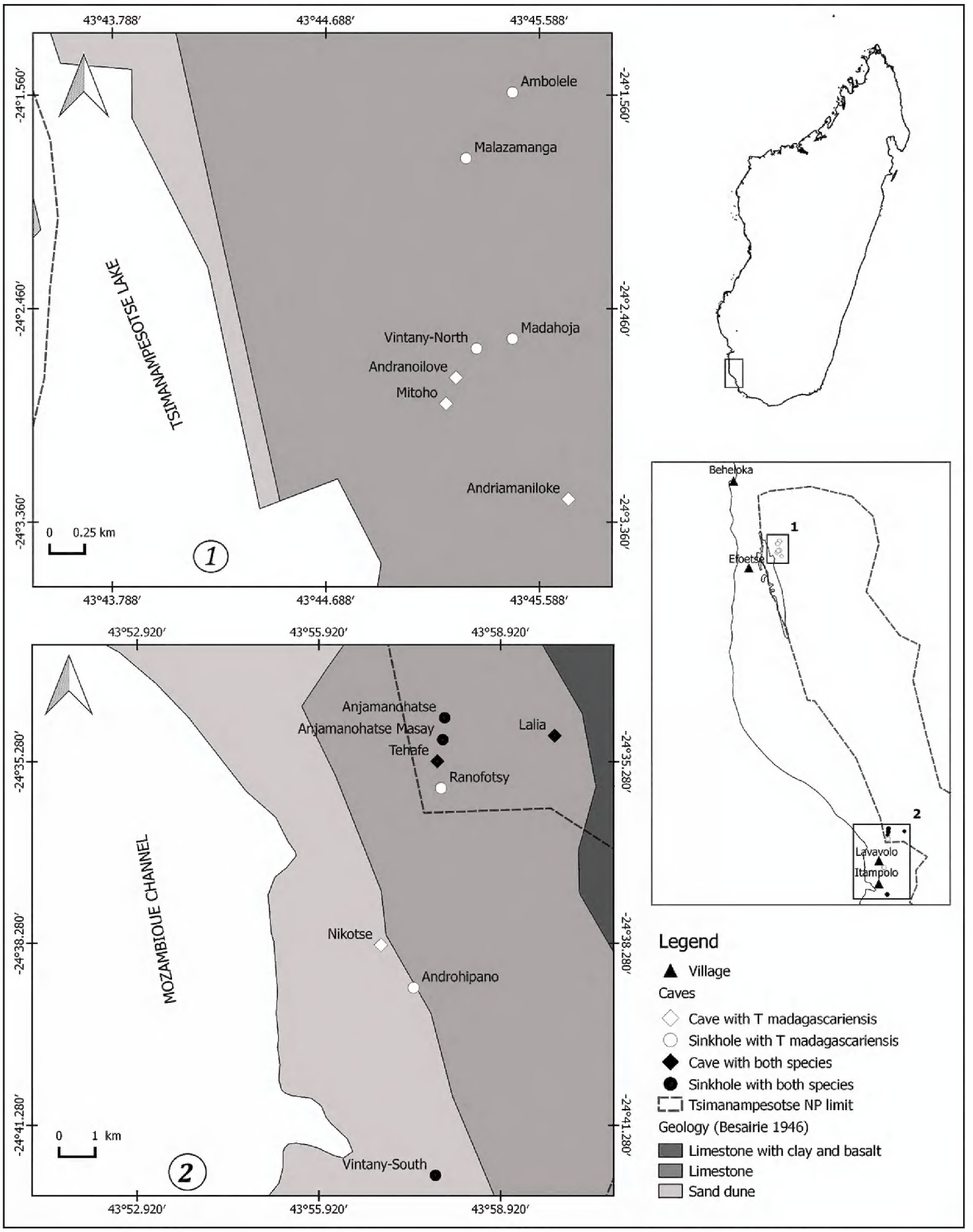

Figure 1. Study area and survey sites for *Typhleotris* spp.

Due to the large sample sizes, only subsamples of *T. madagascariensis* were measured for total length (TL) and standard length (SL) to the nearest 0.1 millimetre with a Vernier calliper, weighed to the nearest 0.1 gram using a 500 g Maul pocket scale (Baeck et al. 2013). After the measurements all animals were released in their natural habitat after having been marked.

Data analyses

The Fulton's condition index was calculated as a measure of body condition for each individual with the formula $K = 100^* W / TL^3$, where W is the body mass of the fish in grams and TL is the length of the fish in centimetre (Nielsen and Johnson 1983).

Statistical analyses were performed in R 3.0 and SPSS 22.0 for WINDOWS. Wilcoxon matched-pairs signed rank test was used to assess differences between seasons (dry and wet seasons). Mann-Whitney-U test was used to compare the abundance and condition of *T. madagascariensis* and the abiotic factors of the waters where *T. mararybe* was present against the waters where *T. mararybe* was absent. Correlations between water characteristics and fish abundances and body condition were calculated by Spearman rank correlations.

Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility at http://www.gbif.org/dataset/937bb0a3-e9e0-400a-8e07-0b33176953aa.

Dataset citation provided by publisher: Rasoloariniaina, J.R., 2015. SuLaMa blind cave fish occurence data. SuLaMa - Participatory research to support sustainable land management on the Mahafaly Plateau in southwestern Madagascar. doi: 10.15468/dluigi.

Results

Water quality

The mean values of the physicochemical parameters of subterranean waters are shown in Table 1. Values are means of two to four measurements per water body. The water temperature ranged from 26.35° to 29.08°C (median = 27.75°C) during the dry season and from 26.87° to 30.03°C (median = 27.90°C) during the rainy season. The pH values ranged from 7.19 to 7.68 (median = 7.32) during the dry season and from 7.04 to 7.67 (median = 7.35) during the rainy season. The dissolved oxygen ranged from 4.85 to 7.85 mg/l (median = 5.35 mg/l) during de dry season and from 2.10 to 6.58 mg/l (median = 4.00 mg/l) during the rainy season. The electrical conductivity (EC) varied from 1448.50 to 3043.00 µS/cm during the dry season (median = 1741.25 µS/cm) and from 979.00 to 3121.25 (median = 1665.00 µS/cm) during the rainy season.

The ammonium content ranged from 0.23 to 1.94 mg/l (median = 0.29 mg/l) during the dry season and from 0.17 to 24.62 mg/l (median = 0.56 mg/l) during the rainy season. The nitrate concentration ranged from 0.00 to 4.55 mg/l (median = 0.00 mg/l) during the dry season and from 0.00 to 5.40 mg/l (median = 0.00mg/l) during the rainy season. The nitrite concentration ranged from 0.00 to 9.00 mg/l

Table 1. Number of *Typhleotris* spp. caught during 4 days * 3 hours (= 12 hours) of inventories, their body condition and abiotic characteristics of different water bodies along the Mahafaly Plateau. Values for abiotic conditions are means based on 1-4 measurements per season. For *T. madagascariensis* and *T. mararybe* body condition indices *K* were calculated for ***N*** = 828 and 70 individuals for the dry season and *N* = 1011 and 31 individuals for the rainy season, respectively.

Site	Season	*T. madagascariensis*		*T. mararybe*		Temp (°C)	pH	O_2 (mg/l)	O_2 (%)	EC (µs/cm)	TDS (ppm)	NH_4^+ (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	PO_4^{3-} (mg/l)	Fe (mg/l)
		Abund	*K*	Abund	*K*											
Andranoilove (Cave)	Dry	571	0.98			28.68	7.28	5.25	14.03	3020.50	1508.25	0.24	0.00	1.00	19.35	0.06
	Rainy	645	1.08			30.03	7.04	2.10	5.55	3121.25	1560.25	24.62	2.00	2.50	41.80	0.08
Andriamaniloke (Cave)	Dry	264	0.84			28.73	7.26	5.45	14.63	3043.00	1521.50	0.23	0.00	0.50	14.05	0.10
	Rainy	153	0.97			29.10	7.12	3.15	8.48	3025.75	1512.50	0.17	0.00	3.00	28.00	0.21
Anjamanohatse (Sinkhole)	Dry	5	0.87	2	0.95	26.60	7.38	5.20	13.17	1565.33	782.67	0.37	0.00	7.00	1.90	0.06
	Rainy	3	0.74	1	0.91	27.05	7.29	4.10	10.35	1505.00	752.00	0.41	0.00	2.50	38.95	1.32
Anjamanohatse Masay (Sinkhole)	Dry	2	0.85	13	1.01	26.35	7.32	4.85	12.33	1741.25	869.50	0.29	0.00	0.00	12.10	0.01
	Rainy	0	NA	12	1.01	26.87	7.35	3.90	10.03	1665.00	665.67	0.78	0.00	0.50	47.40	1.27
Lalia (Cave)	Dry	190	0.70	53	0.89	28.05	7.52	7.23	19.18	1488.25	743.75	0.26	0.00	0.00	5.50	0.00
	Rainy	154	0.79	24	1.00	28.33	7.61	6.58	20.45	1452.50	726.50	0.84	0.10	3.00	42.15	0.04
Mitoho (Cave)	Dry	192	0.88			29.08	7.26	5.33	14.50	3015.50	1509.00	0.26	0.00	1.50	23.45	0.10
	Rainy	290	0.99			29.05	7.15	3.48	9.03	2998.25	1499.00	0.32	0.00	2.50	41.50	0.15
Nikotse (Cave)	Dry	1	1.15			27.28	7.29	6.45	16.90	1582.80	785.60	0.41	4.40	2.67	6.37	0.09
	Rainy	0	NA			27.50	7.53	4.00	10.50	1157.00	579.00	0.40	0.00	0.00	21.25	0.73
Ranofotsy (Sinkhole)	Dry	8	0.78			27.55	7.33	5.35	14.00	1900.00	949.50	0.95	0.10	0.00	4.00	0.01
	Rainy	11	0.64			27.90	7.37	5.15	12.85	1791.50	895.00	0.57	0.00	10.00	26.70	0.20
Tehafe (Cave)	Dry	41	0.95	9	1.02	27.75	7.19	5.00	13.25	1740.75	870.50	0.32	4.55	9.00	11.70	0.03
	Rainy	44	0.90	23	0.97	27.48	7.45	4.57	11.57	1050.50	532.75	3.94	3.40	2.00	36.80	0.64
Vintany North (Sinkhole)	Dry	94	0.75			28.83	7.35	5.37	13.97	3015.33	1508.00	0.24	1.00	4.50	46.60	0.10
	Rainy	84	0.76			29.20	7.24	4.58	11.98	3007.75	1503.25	0.26	5.40	5.00	53.20	0.13
Vintany South (Sinkhole)	Dry	1	NA	8	0.84	27.40	7.68	7.85	20.35	1448.50	724.00	1.94	0.65	1.50	35.35	0.05
	Rainy	0	NA	0	NA	27.20	7.67	2.40	5.50	979.00	490.00	0.56	0.00	1.50	26.75	0.95

Abund: Abundance; K: Condition factor; Temp: Temperature, NA: not assessed; O_2 (mg/l): dissolved oxygen; O_2 (%): oxygen saturation; EC = Electric conductivity; TDS: Total dissolved solids

(median = 1.50 mg/l) during the dry season and from 0.00 to 10.00 mg/l (median = 2.50 mg/l) during the rainy season. The phosphorus concentration ranged from 1.90 to 46.60 mg/l (median = 12.10) during the dry season and from 21.25 to 53.20 mg/l (median = 38.95 mg/l) during the rainy season. The iron concentration ranged from 0.00 to 0.10 mg/l (median = 0.06 mg/l) during the dry season and from 0.04 to 1.32 mg/l (median = 0.21 mg/l) during the rainy season.

For both seasons, the abiotic factors showed no significant difference except for the electrical conductivity (as a proxy of salinity) which is significantly higher during the dry season than during the rainy season (Wilcoxon test: W = 28.0, P = 0.017, n = 11).

The pH increased and EC decreased significantly from north to south (r_s = 0.80 for pH and -0.84 for EC, P < 0.01, n = 11; Table 2).

Distribution, abundance and condition of *Typhleotris* spp.

Typhleotris madagascariensis is more widespread and more abundant than *T. mararybe*. *Typhleotris madagascariensis* was found in all eleven water bodies. In contrast, *T. mararybe* was encountered only at five sites, all located in the southern part of the study area (Fig. 1).

The relative abundance of *T. madagascariensis* (as measured by 4 days of 3 hours = 12 hours of capture efforts) varied from 1 to 571 individuals during the dry season and from 0 to 645 individuals during the wet season. The highest numbers were found in the Andranoilove cave in both seasons. The abundance of *T. mararybe* varied from 1 to 53 individuals during the dry season and from 1 to 23 individuals during the wet season. The abundances of neither species differed significantly between seasons (Wilcoxon test: P > 0.05; Table 1).

The condition factor K of *T. madagascariensis* varied from 0.70 to 1.15 during the dry season and from 0.64 to 1.08 during the rainy season. The condition factor of *T. mararybe* varied from 0.84 to 1.02 during the dry season and from 0.91 to 1.01 during the rainy season. There was no seasonal difference in K for either species (Wilcoxon test: P > 0.05; Table 1).

In order to assess correlations between water quality and the abundance and body condition of fish, we pooled the data for the wet and the dry season and calculated annual means for all variables which were then used in Spearman rank correlations. The abundance of *T. madagascariensis* was negatively correlated with the pH of the water body (r_s = -0.76, P = 0.006, n = 11) and positively and significantly correlated with various water characteristics that indicate the concentration of organic matter in the water, such as the electrical conductivity and total dissolved solid material (r_s = 0.78 for both variables, P = 0.005; n = 11; Tables 1, 2). These correlations might represent spurious relationships as the abundance of *T. madagascariensis* decreases significantly from north to south (r_s = -0.78, P < 0.01, n = 11; Table 2), thus following the correlations between geographic latitude, pH and EC (Table 2). Small sample size prohibited multivariate analyses. Iron concentrations were negatively correlated with the abundance

Table 2. Spearman correlation coefficients between the geographic location along a north-south gradient (the lowest number was defined as the northernmost site), abundance and condition of *Typhleotris madagascariensis* (*T. mad.*) and T. *mararybe* (*T. mar.*) and abiotic water characteristics. Oxygen saturation and Total dissolved solids are not shown as they are highly correlated with oxygen concentration and electrical conductivity, respectively. N = 11 for all correlations except for *T. mararybe* where N = 4 or 5. * $p < 0.05$; ** $p < 0.01$.

	Abund. *T. mad.*	**K *T. mad.***	**Abund. *T. mar.***	**K *T. mar.***	**Temp (°C)**	**pH**	**O_2 (mg/l)**	**EC**	**NH_4^+ (mg/l)**	**NO_3^- (mg/l)**	**NO_2^- (mg/l)**	**$PO4^{3-}$ (mg/l)**	**Fe (mg/l)**
Latitude	-0.797**	-0.018	0.100	-0.100	-0.573	.802**	0.555	-0.836**	0.418	0.14	-0.243	-0.491	0.400
Abund. *T. mad.*		0.127	0.700	0.300	0.642*	-0.763**	-0.451	0.779**	-0.100	-0.108	0.3	0.355	-0.706*
K *T. mad.*			-0.200	0.600	0.370	-0.413	-0.515	-0.018	0.055	0.144	-0.299	0.079	0.273
Abund. *T. mar.*				0.500	-0.300	-0.103	0.500	-0.100	0.500	0.41	-0.051	0	-0.900*
K *T. mar.*					-0.200	-0.667	-0.500	0.600	0	-0.154	-0.205	0	-0.100
Temp (°C)						-0.779**	-0.464	0.709*	-0.100	0.21	0.421	0.382	-0.427
pH							0.820**	-0.852**	0.246	0.084	-0.333	-0.337	0.232
O_2^- (mg/l)								-0.627*	0.136	0.331	0.037	-0.318	-0.200
EC									-0.309	-0.252	0.229	0.282	-0.473
NH_4^{+-} (mg/l)										0.443	0.037	-0.009	-0.173
NO_3^{--} (mg/l)											0.242	0.135	-0.243
NO_2^{--} (mg/l)												0.027	-0.197
$PO4^{3-}$ (mg/l)													-0.155

of *T. madagascariensis* (r_s = -0.71, P = 0.017; n = 11). The relationships are not linear but indicate thresholds below which the concentration of the component in question has a limiting effect and upper values beyond which any increase does not result in an increase of fish abundances (Fig. 2). Abundances of *T. mararybe* were also correlated negatively with the concentration of iron (r_s = -0.90, P = 0.037; n = 5).

Body conditions of *T. madagascariensis* and *T. mararybe* were uncorrelated with the abiotic measurements of water quality.

Interspecific relationships

The abundances of the two species of *Typhleotris* spp. were positively correlated (r_s = 0.96, P = 0.011, n = 5; based on annual means of abundances per site). Abundances of *T. madagascariensis* did not differ between sites where *T. mararybe* was present or not (Mann-Whitney-U test: P > 0.05 for both seasons and the annual mean abundance).

During both seasons, the body condition (K) of *T. madagascariensis* was uncorrelated with the body condition of *T. mararybe* and did not differ between sites where *T. mararybe* was present or not (Mann-Whitney-U test: P > 0.05 for both seasons and the annual mean abundance).

Discussion

The distribution, ecological requirements and interspecific interactions of the blind cavefish of Madagascar are poorly known. Recently, a new species of *Typhleotris*, *T. mararybe*, was described from the Mahafaly Plateau which occurs in sympatry with *T. madagascariensis* previously assumed to be the only species present in the area (Sparks and Chakrabarty 2012). Given the morphological similarity of the two species, the questions arises how the two species can coexist in a seemingly poorly structured environment that seems to offer few options for ecological niche separation. In order to come to a better understanding of the interactions of the species and their ecological requirements, we investigated the ranges of *T. madagascariensis* and *T. mararybe*, related their occurrence to physical and chemical water characteristics, and investigated whether or not there was any evidence for competition between the two species as indicated by changes in morphology in the case of sympatry versus allopatry.

Typhleotris mararybe is a sister taxon to *T. madagascariensis* (Chakrabarty et al. 2012). The former species inhabits the southern part of the Mahafaly Plateau (about 10 km around Itampolo) while *T. madagascariensis* is known from Ambilailalike (northeast of Beheloka) to Vintane (2 km south of Itampolo). *Typhleotris madagascariensis* reach higher abundances than *T. mararybe* and are very abundant in dark caves such as Andranoilove, Andriamaniloke and Lalia. Abundances of neither species, varied significantly between seasons, indicating sedentary populations.

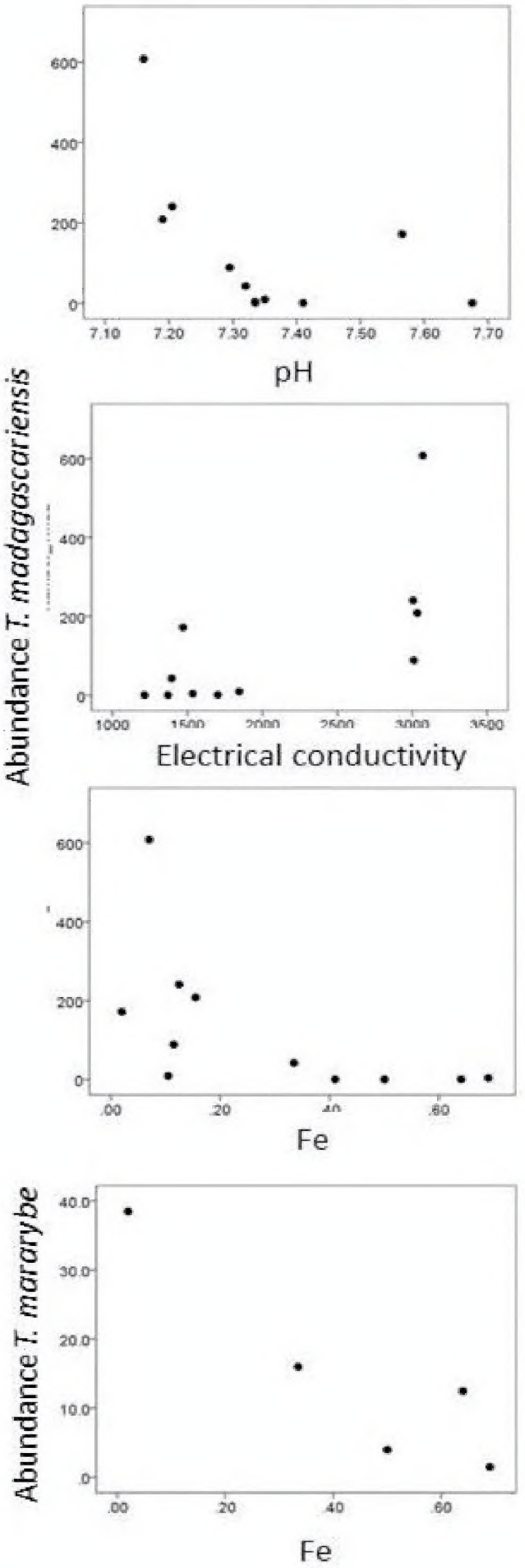

Figure 2. Significant relationships between the abundance of *Typhleotris madagascariensis* and *T. mararybe* and water characteristics.

Water quality

In the groundwater system of the Mahafaly Plateau water quality did not differ between the wet and the dry season. The low annual precipitation in this area of about 400 mm (Ratovonamana et al. 2013) and the feeding of the underground water bodies through subterranean influx over long distances (Guyot 2002, Dworak 2014) does not lead to any relevant change in water quality between seasons.

Analyses of the associations of the two cave fish species are hampered by several boundary conditions. First, the water characteristics (pH, electrical conductivity, total dissolved solids) that are correlated best with the abundance of *T. madagascariensis* are also the variables that show a linear relationship with latitude, increasing or decreasing from north to south. Since the abundances of *T. madagascariensis* co-vary with latitude and these water characteristics, it remains unclear whether the correlations between abundances and water characteristics are simply a consequence of latitudinal variation or whether they represent confining conditions. Furthermore, the relationships between water characteristics and abundances of *T. mararybe* are also difficult to evaluate due to small sample size and the low abundances. Yet, abundances of both species are negatively correlated with the concentrations of iron components on the regional scale (in case of *T. madagascariensis*) as well as on a local scale (in case of *T. mararybe*). Iron is moderately toxic for fish and some of the water bodies come close to inhibiting iron concentrations when compared to other fish species (Shuhaimi-Othman et al. 2015).

Similarly to many other troglobitic species (Poulson 2010), *T. madagascariensis* and *T. mararybe* are not exigent in terms of water quality. Our results indicate that most of the various water physico-chemical parameters recorded for the caves and sinkholes are within the tolerances for fish wellbeing. In caves and sinkholes, the water temperature is relatively high. According to Delince (1992) 30–35 °C is tolerable to fish. The pH was also within the optimal range for fish which is between 6.5 and 8.5 (Nisbet and Verneaux 1970, Boyd 1979). Fish can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 and death is almost certain at a pH below 4.0 or above 11.0 (Ekubo and Abowei 2011). Electrical conductivity is an index of the total ionic content of water, and therefore indicates salinity (Ogbeibu and Victor 1995). EC of freshwater varies between 50 to 1500 µS/cm (Boyd 1979) and seawater has a conductivity around 52,000 µS/cm (Guyot 2002, Rasoloariniaina et al. 2015). Stone and Thomforde (2004) recommended the suitable range for freshwater species of 100–2000 µS/cm. The measured water electrical conductivity during this study was within this range.

Compared with surface lakes, water quality at our sites does not seem ideal for fish development especially with respect of the dissolved oxygen (DO), the nitrogen content and the phosphorus concentrations. The adequate DO value for tropical fish is 5 mg/l (Bhatnagar and Devi 2013). The measured DO of the underground lakes is slightly below this limit during the rainy season (4 mg/l). The amount of oxygen that can be dissolved in water decreases with increasing temperature. The high nitrogen content is an indicator of organic pollution (Abdel-Rahman 2002, Jameel and Siraju-

deen 2006). Ammonia is toxic for fish but the toxicity depends on the water temperature and pH (Arrignon 1976). For aquatic organisms, the maximum limit of ammonia concentration is 0.1 mg/l (Meade 1985, Santhosh and Singh 2007). The ammonia content of the sampled waters was above this value during both seasons (0.29 mg/l during the dry season and 0.56 mg/l during the rainy season). Nitrate is scant in the study area (median = 0 mg/l during both seasons). However, some water bodies had high concentration (up to 5.40 mg/l). Yet, this does not represent a major problem for fish because nitrate is relatively nontoxic to fish and causes health hazard only at high levels above 90 mg/l (Stone and Thomforde 2004). Nitrite is also toxic for fish but the toxicity varies from one species to another. Recommended value should not exceed 0.2 mg/l in freshwater (OATA 2008, Bhatnagar and Devi 2013). The nitrite concentration of the groundwater is largely above these values (1.50 mg/l during the dry season and 2.50 mg/l during the rainy season). Because of accumulation of bat guano in some caves, the nitrogen content of the water was largely above the limit. From a management perspective, the use of some sinkholes for cattle watering worsens the nitrogen sewage. Phosphate is not directly harmful for fish. However, high phosphorus concentration permits excessive growth and reproduction of the algae and constitute a possible source of problem for fish due to the production of toxic substances or oxygen shortage caused by decomposition of organic matter (Arrignon 1976). Phosphate levels of about 0.06 mg/l are desirable for fish culture (Stone and Thomforde 2004). Probably because of the high concentration of phosphorus (up to 53.20 mg/l), different species of alga covered large surface areas of some of the sinkholes. The erosion of phosphate from rocks in the study area seem to be the main origin of this phosphate contamination (Rasoloariniaina et al. 2015).

Interspecific relations between *T. mararybe* and *T. madagascariensis*

In the southern part of the Mahafaly Plateau, the two cavefish species coexist in the groundwater system. In the field, no direct competition was observed between the two species. Furthermore, the abundance and the condition factor of *T. madagascariensis* did not differ significantly between sites where *T. mararybe* was present or not. Though subterranean habitats are nutrient-poor (David et al. 2009) and food availability is likely to be lower than in surface habitats (Poulson 2010), interspecific resource competition seems very low or even absent. This matches the findings by Poulson (2010) who has noticed that competition does not seem to be intense for cavefish. This is surprising as caves represent a stable ecosystem that should allow species to increase their population size up to the carrying capacity. Under these conditions, competition ought to be intense and species occupying the same niches should not be able to coexist, based on Gause's competitive exclusion principle (Gause 1934). From a human perspective, the underground water system seems to offer few options to adapt to different niches. Possible options are excretions from bats, an occasional cockroach dropping into the water, some crustaceans and material blown into the cave from the

cave entrance. Yet, this certainly leaves the question open which resources actually limit cavefish populations (Berti and Messana 2010).

Cave fishes are larger than most other stygobionts and tend to be at the top of subterranean food webs (David et al. 2009). The suggested diet of *T. madagascariensis* consist of either an aquatic micro fauna of epigean origin or plankton or more likely cave crustacean and insects (Berti and Messana 2010). A wide variety of organisms are found in subterranean habitats for example, the associated aquatic fauna of the Mitoho cave crustaceans (decapod *Typhlopatsa pauliani*, isopod *Anopsilana poissoni* and copepods: *Tropocyclops confinis* and *Diaptomus* sp.) and insects (Tanaidaceae *Microvelia mitohoi*) (Decary and Kiener 1970, Remillet 1973, Berti and Messana 2010). Because of the sun exposure, sinkholes are colonized by surface aquatic flora and fauna. This makes this ecosystem richer in terms of food availability with many invertebrate taxa found in open freshwater (dragonflies, water beetles, snails). Crustacean and insect larvae and/or adults from the surface habitats may provide additional resources for cavefish. Yet, despite the seemingly marked ecological differences between water bodies, such as sinkholes and caves, none of these variables seem to have a pronounced impact on the two cavefish species. This might either be a consequence of small sample size or could indicate high ecological tolerances that permitted the species to survive in the region for many millions of years.

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